Charting a Course Toward Diagnostic Monitoring
Appendix 1.

New coral reef attributes supplementing the review by Jameson et al. (1998). References in bold specifically mention the metric potential of the attribute, those in plain text are primary literature which supports the utility of the attribute.

Attribute	Protocol	Region	References
Coral population colony size structure	Colony size frequencies of coral populations can be modeled by log normal distributions. Under "normal" conditions, colony size structure is skewed to the right, with high frequencies of small coral colonies. Evidence from a comparison of coral colony size frequencies from degraded and "less degraded" reefs suggests that under deteriorating environmental conditions, modal coral colony size increases, indicating changes in mortality and recruitment patterns that result in relatively fewer small and more large coral colonies.	Caribbean Pacific Indian	**Bak and Meesters, 1998**
Coral morphology triangles	Adapted from a terrestrial plant ecology methodology, this technique classifies coral reefs according to their conservation value using r-K-S (ruderal/competitor/ stress-tolerator) ternary diagrams based upon the relative abundance of standardized coral morphology categories on each reef. Technique has been calibrated for Indonesian reefs, and assigns a conservation value to each reef based upon its position in an r-K-S ternary diagram. Has the advantage that it does not require coral taxonomic knowledge, but instead utilizes the considerable database of life forms transect data which is commonly collected in monitoring programs of many Indo-Pacific countries.	Caribbean Pacific Indian	**Edinger and Risk, 1999**
Coral fecundity, fertilization rate	Recent results from the large-scale ENCORE experiment show conclusively that increased ammonium and phosphate levels in reef environments have strongly negative effects on coral fecundity and fertilization rate. In experiments with several acroporid species, corals subject to increased nutrient levels had significantly smaller and fewer eggs and less testes, and fertilization rates were reduced. Though the authors did not suggest that these coral parameters be used as a bioassay of eutrophication, these results corroborate earlier suggestions that coral fecundity and fertilization rate may be used as sensitive biocriteria.	Caribbean Pacific Indian	Ward, 1997 Ward and Harrison, 1999
Coral settlement rate	Further results from the ENCORE experiment show that settlement tiles placed in reef environments subject to increased levels of ammonium and of ammonium and phosphate have significantly reduced settlement of coral spat. Though not yet developed into a biomonitoring protocol, use of settlement tiles for water quality monitoring of nutrient inputs to reef environments is a promising technique worthy of further biocriteria research.	Caribbean Pacific Indian	Ward and Harrison, 1997 Ward and Harrison, 1999

Bioaccumulation in sponges	The efficient filter feeders and lipid rich common sponges *Chondrilla nucula* and *Aplysina fistularis* are used as coral surrogates to monitor chemical contaminants in the EPA coral disease survey in the Florida Keys National Marine Sanctuary.	western Atlantic (Florida)	**D. L. Santavy**, U.S. EPA Office of Research and Development, Gulf Ecology Division, pers. com.
Giant clam zooxanthellae	Giant clam zooxanthellae populations are generally considered to be N-limited. Results from the ENCORE experiment demonstrate conclusively that zooxanthellae in *Tridacna maxima* show a number of interrelated responses to increased ammonium, including an increase in the density and chlorophyll content of zooxanthellae, a decrease in the average size of zooxanthellae, and a decrease in the starch sheath surrounding the pyrenoid of the zooxanthellae chloroplasts. This sensitive response of giant clam zooxanthellae populations make them an excellent candidate for development as bioindicators of nutrient enrichment. Monitoring the size of clam zooxanthellae seems particularly promising, as it is quick, easy and does not harm the clam.	Pacific Indian	**ENCORE team, in review** Ambariyanto and Hoegh-Guldberg, 1996 Ambariyanto, 1996 Belda et al., 1993b Belda-Baillie et al., 1998
Giant clam shell growth rate	Further results from the ENCORE experiment show that giant clams (*T. maxima*) exposed to increased levels of ammonium have significantly increased shell growth rates. This parameter is easy and inexpensive to monitor, and with proper calibration could be an excellent biocriteria for monitoring programs concerned with nutrient enrichment.	Pacific Indian	Ambariyanto, 1996 Belda et al., 1993a **ENCORE team, in prep**
Coral Damage Index	Sites are listed as "hot spots" (in need of management attention) if in an transect the percent of broken coral colonies is greater than or equal to 4% or if the percent cover of coral rubble is greater than or equal to 3%.	Red Sea	**Jameson et al., 1999**
Vibrio shiloi as causative agent of *Oculina patagonica* bleaching	Studies using the coral *Oculina patagonica* have linked coral bleaching with a bacterial disease caused by *Vibrio shiloi.* The disease can be blocked by antibiotics. Elevated seawater temperature is a critical factor for this disease. From 16-20°C the disease does not occur, whereas from 25-30°C even a few *Vibrio shiloi* can cause the disease. Increased temperature without the bacteria is insufficient to cause bleaching because antibiotics prevent the bleaching even at elevated seawater temperatures. Elevated temperature triggers bacterial adhesion to coral surface and allows infection to proceed.	Mediterranean coast of Israel.	Rosenberg and Loya, 1999
Coral stress using gene expression	Uses recent advances in molecular biology to visualize changes in scleractinian mRNA abundance. Stressor-specific probes for mRNA are being developed for quantifying the intensity of stress in corals and diagnosing the most likely stressors. Transplantation experiments will be conducted to examine how stressors in natural populations induce gene expression.	western Atlantic (Florida)	**Snell, in progress**

FoRAM (Foraminifers in Reef Assessment Monitoring)	FoRAM consists of a three tiered protocol. Number of tiers used depends on the region being assessed and questions being asked. 1. Sediment constituent analysis, which can address questions of historical change and reference-site suitability. 2. Analysis of live larger foraminiferal assemblages, which can indicate the suitability of sites for organisms with algal symbionts. 3. Analysis of *Amphistegina* populations, including abundance, presence of bleaching, and other evidence of specific stressors to which these foraminifers respond similarly to corals.	western Atlantic (Florida)	**Hallock, 1996** **Cockey et al., 1996** **Hallock, 2000**
Molecular Biomarker System (MBS)	Uses a MBS that assays specific cellular and molecular parameters, to assess the physiological status of coral challenged by heat stress. The MBS distinguished the separate and combined effects of heat and light on the two coral symbionts, a scleractinian coral and a dinoflagellate algae (zooxanthellae). This technology aids in the accurate diagnosis of coral condition because each parameter is physiologically well understood. The MBS technology is reportedly relatively inexpensive, easy to implement, precise, and can be quickly adapted to a high-throughput robotic system for mass sample analysis.	western Atlantic (Florida)	**Downs et al., in press**

Reef Check '97, with notes on recent Reef Check protocol changes	In Reef Check '97 (Hodgson, 1999) twenty-five worldwide and regional "health indicators" were used by trained volunteer recreational divers to provide information about the effects of human activities on coral reefs. This unprecidented effort got hundreds of people out onto reefs using one method to monitor coral reefs and helped raise awareness about coral reefs. The world's oceans were divided into Indo-Pacific, Red Sea and Caribbean (special regional indicators were chosen for biogeographic margins e.g. Arabian Gulf, Hawaii and the E. Pacific). Sites believed to be least affected by human activities and having the highest percentage of seabed covered by living coral and the highest populations of indicator fish and other invertebrates were selected for monitoring. The protocol included the collection of 4 types of data: a site description; a fish survey; an invertebrate survey and a substrate survey. The underwater surveys were made along the 3 and 10 m depth contours. The following conclusions were drawn from the study. Results showed that no reefs had high numbers of most indicator organisms, suggesting to the author that few, if any, reefs have been unaffected by fishing and gathering. The low percentage cover of pollution indicators was taken to suggest that sewage pollution is not a serious problem a most of the sites (biased toward perceived good condition). Technical recommendations regarding the use of Reef Check for long-term monitoring are given in Hodgson and Stepath (1999). Hodgson (1999) mentions some ways the protocols could be improved (i.e., establishing sample size goals and obtaining historical baseline data). Improvement and refinements in the program are also discussed in Hodgson (2000). We suggest the protocols could also be improved by: 1. Verifying data quality with an analysis of the variation between teams in controlled studies. 2. Confirming that a dose-response change in "health indicator" value is reliable, interpretable and not swamped by natural variation; 3. Sampling across a gradient of human influence rather than relying on the perception of participants to select monitoring sites least affected by human activity (or hoping groups will have the time to survey multiple sites representative of moderate and heavy human impacts (Hodgson and Stepath, 1999; Reef Check, 2000)); 4. Classifying sites (before monitoring begins) with respect to similar environmental conditions so appropriate sites can be selected to allow valid comparisons among similar sites (site description sheets prepared by teams are used to compare sites after monitoring (Hodgson and Stepath, 1999; Reef Check, 2000); 5. Resurveying the same sites every year (G. Hodgson, Reef Check, pers. comm.) 6. Calibrating indicators or collecting data on fishing effort or pollution to determine the causes of the degradation. Otherwise the causes of presumed changes (degradation) are assumed; 7. Using minimally degraded reference sites to compare against degraded sites (which in Reef Check are biased towards a perceived less impacted condition); and by 8. Not using the Bray Curtis similarity index to examine the relationships among all sites for six worldwide indicators (Hodgson, 1999) because this index has been shown in independent independetests to fail to discriminate among sites (CAO, 1997). independent tests to fail to discriminate among sites (Cao, 1997).	Indo-Pacific, Red Sea, Caribbean	**Hodgson, 1999** **Hodgson and Stepath, 1999** **Hodgson, 2000** **Reef Check, 2000**

Reef Check Coral Reef Health Index (CRHI)	The CRHI was calculated for six indicators (butterflyfish, Haemulidae grouper, *Diadema*, hard corals and lobster) for 269 sites from 3 regions. The highest mean abundance of an organism recorded at any site in the world was used as the maximum possible value to determine a lower, middle and upper third for 269 sites in 3 regions. Then, for each site, a value of 0-3 was assigned for each indicator depending on the mean abundance in comparison to the cut-off levels for each third. Means in the lower, middle and upper third were assigned a value of 1, 2 and 3 respectively while a mean of zero was assigned a zero (except for *Diadema* where the values were reversed as high numbers are considered to be unhealthy). The CRHI was calculated by adding the 6 values together. The maximum possible CRHI is : 6 indicators X 3 = 18. The mean CRHI values from the study were 3.8, 4.0 and 3.5 respectively for the Indo-Pacific, Red Sea and Caribbean regions, out of a maximum possible CRHI of 18. There was no significant difference among the values from the three regions and the low CRHI scores were assumed to indicate how few sites had high numbers of indicators recorded. The comparison among sites could be improved by classifying sites as mentioned in (2) above. Much early freshwater work to detect the influence of human actions on biological systems emphasized abundance (or population size or density) of indicator taxa, often species with commodity value or thought to be keystone species. Generally, however, population size varies too much even under natural unimpaired conditions to be a reliable indicator of biological condition. Population size changes in complex ways in response to changes in natural factors such as food supply, disease, predators, temperature, salinity and demographic lags. In studies to determine environmental impacts, the interaction between variability and the size of the potential impact (effect size) must also be taken into account, because that interaction affects statistical power (Osenberg et al. 1994). High variation in population size, even in natural environments, interacts in complex ways with changes in abundances stimulated by human actions. Thus it can be very difficult to detect and interpret the effects of human actions even with advanced experimental designs. The minimum level of sampling effort may exceed the planning, sampling, and analytical capability of many monitoring situations. By shifting the focus to better-behaved indicators such as changes in taxa richness, loss of sensitive taxa, or changes in trophic organization, it is possible to develop a clearer and broader understanding of biological changes (Karr and Chu, 1999). Using the highest mean abundance of an organism recorded at any site in the world as the maximum reference condition for sites also disregards the effects of regional, seasonal and environmental factors on species abundance and is probably setting the reference bar too high in some areas and too low in others.	Global	**Hodgson, 1999**
Reef Check Distance-Population Index (DPI)	The DPI was calculated by assigning a score for both population of nearest city and the distance to that city as follows: Population 0-10,000 = 0; 10,000-50,000 = 1; 50,000-100,000 = 2; > 100,000 = 3. Distance > 50 km = 0; 25-49 km = 1; 10-24 km = 2; 0-9 km = 3. The DPI was then calculated as the sum of the population size and distance scores. The higher the index means the site is close to a dense population. The maximum DPI is 6. The CRHI was plotted versus the DPI to show that a sizable number of sites located far from population centers had a low health index. See comments above regarding the applicability of the CRHI.	Indo-Pacific, Red Sea, Caribbean	**Hodgson, 1999**